

IN THE SPECIFICATION:

Please replace the paragraph beginning on Page 1, line 9, with the following:

Differences in various phenotypes of humans, including disease, are known to be derived from the differences in DNA nucleotide sequence in an individual genome. This difference is called single nucleotide polymorphism (SNP). SNPs are found widely in human genome of about 3 billion base pairs and the total number of SNPs is not less than 3 millions. Thus, SNPs can be the DNA markers having exceptionally higher density than known DNA markers such as RFLP (restriction fragment length polymorphism) and STR (microsatellite), which are conventional DNA markers. Therefore, high-precision analysis, which has been impossible using conventional DNA markers, is possible by using SNPs, and it is hoped that SNP can be applied to detection of disease genes, determination of disease sensitivity, and development of pharmaceutical.

Please replace the paragraph beginning on Page 2, line 11, with the following:

However, there is a problem with this technique because the region for searching is from 200 to 300 bp and the detection fails when the region is not more than that.

Please replace the paragraph beginning on Page 3, line 18, with the following:

The present inventors have intensively been studying homologous recombination of DNA *in vivo*. As a result of the study, the present inventors revealed that *E coli* RecA protein involved in homologous recombination can make triple strand DNA without long

homologous region and that the triple strand DNA becomes unstable by heat when a pair of mismatch exists in one double strand DNA among triple strand DNA. Then, the present inventors reminded a following method for detecting DNA polymorphism. First, an oligonucleotide probe complementary to one strand of test DNA region to examine is prepared and hybridized to the test DNA region using a homologous recombination protein. After the formation of triple strand DNA in the test DNA region, the homologous recombination protein is removed. When polymorphism exists in the test DNA region, mismatch nucleotide pair occurs between the oligonucleotide probe and one strand of the test DNA region which makes the structure of triple strand DNA unstable to heat compared to the test DNA region in which no polymorphism exists. If the triple strand DNA is treated with heat, the oligonucleotide probe is released from unstable triple strand DNA in which mismatch exists. Therefore, the present inventors considered that the existence of polymorphism in test DNA region could be detected by detecting oligonucleotide probe hybridized to the test DNA region.

Please replace the paragraph beginning on Page 4, line 6, with the following:

The present inventors investigated whether the detection of DNA polymorphism was actually possible by using the above-mentioned method. At first, the present inventors examined the condition in which a test DNA region including polymorphism and an oligonucleotide which was a probe for the detection of polymorphism could form stable triple strand DNA through a homologous recombination protein. As a result, it was

revealed that triple strand DNA could be formed if the length of the oligonucleotide probe was not less than 40 nucleotides. Next, the present inventors examined the stability of triple strand DNA, which comprises an oligonucleotide completely complementary to one strand of a test DNA region or an oligonucleotide including one mismatch and the test double DNA, to heat. As a result, the triple strand DNA formed when the completely complementary oligonucleotide was more stable than that containing an oligonucleotide including one mismatch. Thus, the difference of thermostability between them was clear. It was revealed that the stability of the triple strand DNA was markedly affected by mismatch derived from even one nucleotide mutation in a DNA strand. Therefore, it is considered that the heat treatment of triple strand DNA makes oligonucleotide having mismatch release from target DNA and makes the structure of triple strand DNA collapse. Using the above-mentioned result, precise detection of polymorphism existing in the test DNA is possible by detecting oligonucleotide that forms triple strand DNA with target DNA even after heat treatment of the triple strand DNA that is formed using homologous recombination protein.

**Please replace the paragraph beginning on Page 5, line 11, and
ending on Page 6, line 21 with the following:**

The present invention relates to a new method which does not require long DNA region for searching and can detect DNA polymorphism with high specificity and efficiency. More specifically, the present invention provides the following:

[1] A method for detecting a DNA polymorphism in a double strand DNA, said method comprising the steps of (a) to (d) below:

- (a) contacting (i) a double strand DNA comprising a test polymorphic site, (ii) an oligonucleotide probe that hybridizes to a region comprising said polymorphic site in said double strand DNA, and (iii) a homologous recombination protein under reaction conditions where a triple strand DNA complex is formed,
- (b) removing the homologous recombination protein from the triple strand DNA complex formed in the step (a), thereby generating a triple strand DNA,
- (c) conducting heat treatment of the triple strand DNA generated by removing the homologous recombination protein, under conditions where the oligonucleotide probe is released from said triple strand DNA, when the test polymorphic site in the double strand DNA is not complementary to a corresponding site in said oligonucleotide probe,
- (d) detecting an oligonucleotide probe that binds to the double strand DNA to form the triple strand DNA,

[2] The method of [1], wherein the double strand DNA comprising a test polymorphic site has a DNA terminus,

[3] The method of [2], wherein the test polymorphic site is located within 20 bases from the DNA terminus,

[4] The method of [1], wherein the length of the oligonucleotide probe is from 20 to 120 bases,

[5] The method of [1], wherein the homologous recombination protein is a RecA protein from *E. coli*,

[6] The method of [1], wherein, in the step (a), a nucleotide triphosphate is added to the reaction system,

[7] The method of [1], wherein, in the step (b), the homologous recombination protein is removed by conducting protein degradation enzyme treatment,

[8] The method of [7], wherein the protein degradation enzyme is proteinase K,

[9] A kit for detecting a polymorphism in a double strand DNA comprising a test polymorphic site, said kit comprising the following components: (a) an oligonucleotide probe that hybridizes to the double strand DNA comprising the test polymorphic site and (b) a homologous recombination protein,

[10] A kit of [9], further comprising at least one selected from the group consisting of (i) a reagent removing the homologous recombination protein, (ii) nucleotide triphosphate, and (iii) a buffering agent.

Please replace the paragraph beginning on Page 6, line 24, with the following:

Figures 1A-1C show the following: Figure 1A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide containing sequence complementary to the DNA. Figure 1B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA

structure was electrophoresed. Figure 1C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane M: DNA size marker (The left in the figure indicates size. This size marker is λ DNA which was cut by restriction enzyme Hind III and whose 5'-terminal was labeled with ^{32}P using T4 Polynucleotide kinase and $[\gamma\text{-}^{32}\text{P}]$ ATP.

Please replace the paragraph beginning on Page 7, line 17, with the following:

Figures 2A-2C show the following: Figure 2A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide containing sequence complementary to the DNA. Figure 2B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. Figure 2C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figure 1B.

Please replace the paragraph beginning on Page 7, line 34, with the following:

Figures 3A-3C show the following: Figure 3A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide containing sequence complementary to the DNA. Figure 3B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA

structure was electrophoresed. Figure 3C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figure 1B.

Please replace the paragraph beginning on Page 8, line 24, with the following:

Figures 4A-4C show the following: Figure 4A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide containing sequence complementary to the DNA. Figure 4B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. Figure 4C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figure 1B in which labeled oligonucleotide 11 that had the 5'-terminal sequence extending 20 per of oligonucleotide 1 was used.

**Please replace the paragraphs beginning on Page 9, line 17 and ending
at Page 11, line 20 with the following:**

Figures 6A-6C show the following: Figure 6A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide containing sequence complementary to the DNA. "G" or "C" on the oligonucleotide indicates the types of the nucleotide located at corresponding base pair of the target DNA

shown just described above in character in the figure. Figure 6B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. Figure 6C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The heat treatment was conducted at 25°C for 10 minutes using oligonucleotide 1.

Lane 2: The reaction was performed in the same manner of lane 1 in which the heat treatment was conducted at 65°C for 10 minutes.

Lane 3: The reaction was performed in the same manner of lane 1 in which the heat treatment was conducted at 70°C for 10 minutes.

Lane 4: The reaction was performed in the same manner of lane 1 in which the heat treatment was conducted at 75°C for 10 minutes.

Lane 5: The reaction was performed in the same manner of lane 1 in which the heat treatment was conducted at 80°C for 10 minutes.

Lane 6: The reaction was performed in the same manner of lane 1 in which the heat treatment was conducted at 85°C for 10 minutes.

Lane 7: The reaction was performed in the same manner of lane 1 in which the heat treatment was conducted at 90°C for 10 minutes.

Lane 8: The reaction was performed in the same manner of lane 1 in which the heat treatment was conducted at 95°C for 10 minutes.

Lane 9: The reaction was performed in the same manner of lane 1 in which oligonucleotide 16 was used.

Lane 10: The reaction was performed in the same manner of lane 2 in which oligonucleotide 16 was used.

Lane 11: The reaction was performed in the same manner of lane 3 in which oligonucleotide 16 was used.

Lane 12: The reaction was performed in the same manner of lane 4 in which oligonucleotide 16 was used.

Lane 13: The reaction was performed in the same manner of lane 5 in which oligonucleotide 16 was used.

Lane 14: The reaction was performed in the same manner of lane 6 in which oligonucleotide 16 was used.

Lane 15: The reaction was performed in the same manner of lane 7 in which oligonucleotide 16 was used.

Lane 16: The reaction was performed in the same manner of lane 8 in which oligonucleotide 16 was used.

Lane 17: After the reaction mixture including 1 pmol labeled oligonucleotide 1, 10 pmol unlabeled oligonucleotide 2, 100 ng M13 mp18 ssDNA, 4.8 mM ATP- γ S, 30 mM Tris acetate (pH 7.2), and 20 mM magnesium acetate was incubated at 37°C for 30 minutes, 0.5% (W/Vol) SDS and 0.7 mg/ml proteinase K was added to the mixture. Then,

the mixture was incubated at 37°C for 30 minutes. Subsequent reaction was performed in the same manner of lane 1.

Lane 18: The reaction was performed in the same manner of lane 17 in which the reaction mixture was treated with heat at 65°C for 10 minutes.

Lane 19: The reaction was performed in the same manner of lane 17 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 20: The reaction was performed in the same manner of lane 17 in which the reaction mixture was treated with heat at 75°C for 10 minutes.

Lane 21: The reaction was performed in the same manner of lane 17 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 22: The reaction was performed in the same manner of lane 17 in which the reaction mixture was treated with heat at 85°C for 10 minutes.

Lane 23: The reaction was performed in the same manner of lane 17 in which the reaction mixture was treated with heat at 90°C for 10 minutes.

Please replace the paragraph beginning on Page 11, line 21, with the following:

Figure 7 shows the intensity of the signal from the labeled oligonucleotide, measured by BAS2000 Image analyzer, and the result. Longitudinal axis indicates the temperature

(°C). Triangles show the result of lane 1 to 8 while black triangles show the result of lane 9 to 16. Circles show the result of lane 17 to 23.

Please replace the paragraph beginning on Page 11, line 28, with the following:

Figure 8 schematically shows the relation of the position between target DNA (PCR product) used for the experiment and the oligonucleotide containing sequence complementary to the DNA. "A", "T", "G" or "C" on the oligonucleotide indicates the types of the nucleotide located at corresponding base pair of the target DNA shown just described above in character in the figure.

Please replace the paragraphs beginning on Page 11, line 34 and ending on Page 15, line 30, with the following:

Figures 9A-9B show the following: Figure 9A is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. Figure 9B is the photograph of staining gel corresponding to Figure 9A with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figures 6B-6C in which PCR product was used as the target DNA, and oligonucleotide 3 was used.

Lane 2: The reaction was performed in the same manner of lane 1 in which oligonucleotide 17 was used.

Lane 3: The reaction was performed in the same manner of lane 1 in which oligonucleotide 18 was used.

Lane 4: The reaction was performed in the same manner of lane 1 in which oligonucleotide 19 was used.

Lane 5: The reaction was performed in the same manner of lane 1 in which the reaction mixture was treated with heat at 70°C for 10 minutes after unused oligonucleotide was removed by S-400 spin column.

Lane 6: The reaction was performed in the same manner of lane 2 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 7: The reaction was performed in the same manner of lane 3 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 8: The reaction was performed in the same manner of lane 4 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 9: The reaction was performed in the same manner of lane 1 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 10: The reaction was performed in the same manner of lane 2 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 11: The reaction was performed in the same manner of lane 3 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 12: The reaction was performed in the same manner of lane 4 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 13: The reaction was performed in the same manner of lane 1 in which the PCR Product (b) that was obtained by the PCR using primer 3 and primer 2 was used as the target DNA.

Lane 14: The reaction was performed in the same manner of lane 13 in which oligonucleotide 17 was used.

Lane 15: The reaction was performed in the same manner of lane 13 in which oligonucleotide 18 was used.

Lane 16: The reaction was performed in the same manner of lane 13 in which oligonucleotide 19 was used.

Lane 17: The reaction was performed in the same manner of lane 13 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 18: The reaction was performed in the same manner of lane 14 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 19: The reaction was performed in the same manner of lane 15 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 20: The reaction was performed in the same manner of lane 16 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 21: The reaction was performed in the same manner of lane 13 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 22: The reaction was performed in the same manner of lane 14 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 23: The reaction was performed in the same manner of lane 15 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 24: The reaction was performed in the same manner of lane 16 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 25: The reaction was performed in the same manner of lane 1 in which the PCR Product (c) that was obtained by the PCR using primer 4 and primer 2 was used as the target DNA.

Lane 26: The reaction was performed in the same manner of lane 25 in which oligonucleotide 17 was used.

Lane 27: The reaction was performed in the same manner of lane 25 in which oligonucleotide 18 was used.

Lane 28: The reaction was performed in the same manner of lane 25 in which oligonucleotide 19 was used.

Lane 29: The reaction was performed in the same manner of lane 25 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 30: The reaction was performed in the same manner of lane 26 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 31: The reaction was performed in the same manner of lane 27 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 32: The reaction was performed in the same manner of lane 28 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 33: The reaction was performed in the same manner of lane 25 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 34: The reaction was performed in the same manner of lane 26 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 35: The reaction was performed in the same manner of lane 27 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 36: The reaction was performed in the same manner of lane 28 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 37: The reaction was performed in the same manner of lane 1 in which the PCR Product (d) that was obtained by the PCR using primer 5 and primer 2 was used as the target DNA.

Lane 38: The reaction was performed in the same manner of lane 37 in which oligonucleotide 17 was used.

Lane 39: The reaction was performed in the same manner of lane 37 in which oligonucleotide 18 was used.

Lane 40: The reaction was performed in the same manner of lane 37 in which oligonucleotide 19 was used.

Lane 41: The reaction was performed in the same manner of lane 37 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 42: The reaction was performed in the same manner of lane 38 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 43: The reaction was performed in the same manner of lane 39 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 44: The reaction was performed in the same manner of lane 40 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 45: The reaction was performed in the same manner of lane 37 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 46: The reaction was performed in the same manner of lane 38 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 47: The reaction was performed in the same manner of lane 39 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 48: The reaction was performed in the same manner of lane 40 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

**Please replace the paragraphs beginning on Page 15, line 31 and
ending at Page 17, line 18, with the following:**

Figures 10A-10C show the following: Figure 10A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide containing sequence complementary to the DNA. "G" on the oligonucleotide indicates the relative position of mutation. Figure 10B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was

electrophoresed. Figure 10C is the photograph of staining gel corresponding to Figure 10B with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figures 6B-6C of Example 6 in which oligonucleotide 20 was used.

Lane 2: The reaction was performed in the same manner of lane 1 in which oligonucleotide 21 was used.

Lane 3: The reaction was performed in the same manner of lane 1 in which oligonucleotide 22 was used.

Lane 4: The reaction was performed in the same manner of lane 1 in which oligonucleotide 23 was used.

Lane 5: The reaction was performed in the same manner of lane 1 in which oligonucleotide 24 was used.

Lane 6: The reaction was performed in the same manner of lane 1 in which oligonucleotide 25 was used.

Lane 7: The reaction was performed in the same manner of lane 1 in which oligonucleotide 26 was used.

Lane 8: The reaction was performed in the same manner of lane 1 in which oligonucleotide 27 was used.

Lane 9: The reaction was performed in the same manner of lane 1 in which oligonucleotide 28 was used.

Lane 10: The reaction was performed in the same manner of lane 1 in which ologonucleotide 29 was used.

Lane 11: The reaction was performed in the same manner of lane 1 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 12: The reaction was performed in the same manner of lane 2 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 13: The reaction was performed in the same manner of lane 3 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 14: The reaction was performed in the same manner of lane 4 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 15: The reaction was performed in the same manner of lane 5 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 16: The reaction was performed in the same manner of lane 6 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 17: The reaction was performed in the same manner of lane 7 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 18: The reaction was performed in the same manner of lane 8 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 19: The reaction was performed in the same manner of lane 9 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 20: The reaction was performed in the same manner of lane 10 in which the reaction mixture was treated with heat at 80°C for 10 minutes.

**Please replace the paragraphs beginning on Page 17, line 21 and
ending on Page 18, line 34, with the following:**

Figures 12A-12C show the following: Figure 12A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide containing sequence complementary to the DNA. "C" on the oligonucleotide indicates the relative position of mutation. Figure 12B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. Figure 12C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figures 6B-6C of Example 6.

Lane 2: The reaction was performed in the same manner of lane 1 in which oligonucleotide 30 was used.

Lane 3: The reaction was performed in the same manner of lane 1 in which oligonucleotide 31 was used.

Lane 4: The reaction was performed in the same manner of lane 1 in which oligonucleotide 32 was used.

Lane 5: The reaction was performed in the same manner of lane 1 in which oligonucleotide 16 was used.

Lane 6: The reaction was performed in the same manner of lane 1 in which oligonucleotide 33 was used.

Lane 7: The reaction was performed in the same manner of lane 1 in which oligonucleotide 34 was used.

Lane 8: The reaction was performed in the same manner of lane 1 in which oligonucleotide 35 was used.

Lane 9: The reaction was performed in the same manner of lane 1 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 10: The reaction was performed in the same manner of lane 2 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 11: The reaction was performed in the same manner of lane 3 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 12: The reaction was performed in the same manner of lane 4 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 13: The reaction was performed in the same manner of lane 5 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 14: The reaction was performed in the same manner of lane 6 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 15: The reaction was performed in the same manner of lane 7 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 16: The reaction was performed in the same manner of lane 8 in which the reaction mixture was treated with heat at 70°C for 10 minutes.

**Please replace the paragraphs beginning on Page 18, line 35 and
ending on Page 19, line 32, with the following:**

Figures 13A-13B show the following: Figure 13A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide containing sequence complementary to the DNA. Figure 13B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. Figure 13C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1 and 2: The reaction was performed in the same manner of lane 1 of Figures 1B-1C of Example 1.

Lane 3: The reaction was performed in the same manner of lane 1 in which oligonucleotide 36 that had insertion mutation was used.

Lane 4: The reaction was performed in the same manner of lane 1 in which oligonucleotide 16 that had mismatch mutation was used.

Lane 5: The reaction was performed in the same manner of lane 1 in which oligonucleotide 37 that had deletion mutation was used.

Lane 6: The reaction was performed in the same manner of lane 1 in which the reaction mixture was treated with the heat at 70°C for 10 minutes.

Lane 7: The reaction was performed in the same manner of lane 2 in which the reaction mixture was treated with the heat at 70°C for 10 minutes.

Lane 8: The reaction was performed in the same manner of lane 3 in which the reaction mixture was treated with the heat at 70°C for 10 minutes.

Lane 9: The reaction was performed in the same manner of lane 4 in which the reaction mixture was treated with the heat at 70°C for 10 minutes.

Lane 10: The reaction was performed in the same manner of lane 5 in which the reaction mixture was treated with the heat at 70°C for 10 minutes.

Please replace the paragraph beginning on Page 20, line 7, with the following:

In this invention, "polymorphism" means the individual difference in genome of identical species. The polymorphism containing the difference of a nucleotide is preferred in this invention. Such "polymorphism" includes SNP (single nucleotide polymorphism) in which a nucleotide is inserted or deleted is also included.

**Please replace the paragraph beginning on Page 26, line 11 and
ending on Page 27, line 5, with the following:**

The present inventors revealed that there is the difference in stability to heat between the triple strand DNA in which the test polymorphic site in the target double strand DNA

is complementary to the corresponding site in oligonucleotide probe and in which it is not complementary. When the test polymorphic site in the target double strand DNA is not complementary to the corresponding site in oligonucleotide probe, the oligonucleotide probe (described as "mismatch probe" hereafter) comprising the triple strand DNA can be released by the heat treatment under the suitable condition. The condition of temperature, in which oligonucleotide probe is not released from the triple strand DNA in which the test polymorphic site in the target double strand DNA is complementary to the corresponding site in oligonucleotide probe and is released from the triple strand DNA in which the site is not complementary to the corresponding site, varies depending on the length of target double strand DNA comprising the triple strand DNA, the length of the oligonucleotide probe, and their DNA nucleotide sequences, the extent of the complementation, and composition of reaction mixture (such as the concentration of Tris). Optimal condition (such as composition of reaction mixture and temperature of heat treatment) can be suitably selected according to experiment and experience by one skilled in the art. Specifically, the condition indicated in Example 5 (Figures 6A-6C) can be used. Generally, when the concentration of Tris in the reaction mixture is decreased, the mismatch probe becomes easy to be released from the triple strand DNA. When the concentration of Tris is increased, the mismatch probe becomes hard to be released. Therefore, the concentration of Tris is preferred to be low when using long oligonucleotide probe while the concentration of Tris is preferred to be high when using short oligonucleotide probe.

**Please replace the paragraph beginning on Page 28, line 34 and
ending on Page 29, line 2, with the following:**

The above-mentioned kit of this invention can include nucleotide triphosphate, a buffer agent, and a reagent removing the homologous recombination protein. The reagent removing homologous recombination protein includes proteins such as protein degradation enzymes and such.

Please replace the paragraph beginning on Page 29 line 19, with the following:

Any patents, patent applications, and publications cited herein are incorporated by reference.

**Please replace the paragraph beginning on Page 29 line 27 and
ending on Page 30, line 13, with the following:**

The experiment was conducted to examine reaction components when triple strand DNA was formed. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as target double strand DNA and 60 mer oligonucleotide 1 and 2 that have terminal sequence of the target DNA were prepared. pBR322 DNA cut with restriction enzyme Sca I to make it linear and 60 mer oligonucleotide 3 that has terminal sequence of the target DNA were prepared as target DNA. Oligonucleotide 1, 2, and 3 have the direction of sequence indicated as upper part of Figure 1A. 5'-terminal of oligonucleotide 1 was labeled with ³²P using T4 polynucleotide kinase and [γ -³²P] ATP. Deproteinization was

conducted by incubating 1 pmol labeled oligonucleotide 1, 3.0 μ g RecA protein, 4.8 mM ATP- γ S, and 200 ng target DNA with 20 mM magnesium acetate and 30 mM Tris acetate (pH 7.2) at 37°C for 30 minutes, adding 0.5% (W/Vol) SDS and 0.7 mg/ml proteinase K, and then incubating) at 37°C for 30 minutes. A half of the reaction mixture was electrophoresed with 1% agarose gel. The gel was stained with ethidium bromide, and the photograph of DNA was recorded. Gel was set on filter paper and was dried up in gel dryer. Autoradiogram of the gel was obtained, and signal from labeled oligonucleotide was recorded on X ray film. The result is shown in lane 1 of Figure 1B. The nucleotide sequence of the oligonucleotides used were as follows:

Please replace the paragraph beginning on Page 30, line 20, with the following:

Figure 1C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide.

**Please replace the paragraph beginning on Page 30, line 29 and
ending on Page 31, line 2, with the following:**

We examined the orientation of oligonucleotide sequence necessary for the formation of triple strand DNA. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as target DNA and 60 mer oligonucleotide 1, 2, 4, and 5 that have both terminal sequence of the target DNA were prepared. The oligonucleotide has the orientation of sequence indicated as upper part of Figure 2A. The condition of reaction was same as

Example 1. The result is shown in Figure 2B. The nucleotide sequence of the oligonucleotides used were as follows:

Please replace the paragraph beginning on Page 31, line 7, with the following:

Figure 2C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide.

Please replace the paragraph beginning on Page 32, line 1, with the following:

Figure 3C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide.

Please replace the paragraph beginning on Page 32, line 11, with the following:

The experiment to examine the length of oligonucleotide sequence necessary for the formation of the triple strand DNA was carried out. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as a target DNA and 20-80 MER oligonucleotide that has terminal sequence of the target DNA were prepared. The condition of reaction was same as Example 1. The result is shown in Figure 4B. The nucleotide sequence of the oligonucleotides used were as follows:

Please replace the paragraph beginning on Page 32, line 31, with the following:

Figure 4C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide.

Please replace the paragraph beginning on Page 33, line 6, with the following:

We examined the thermostability of triple strand DNA. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as a target DNA and 60mer oligonucleotide 1 that has terminal sequence of the target DNA were prepared. 5'-terminal of oligonucleotide 1 was labeled with ^{32}P . The reaction mixture contains 1 pmol labeled oligonucleotide 1, 200 ng target DNA, 3.0 μg RecA protein, 4.8 mM ATP- γS , 30 mM Tris acetate (pH 7.2), and 20 mM magnesium. After the reaction mixture was incubated at 37°C for 30 minutes, 0.5 % (W/Vol) SDS and 0.7 mg/ml proteinase K was added to the mixture. Then, the mixture was incubated at 37°C for 30 minutes. After phenol-chloroform extraction was performed once, unused oligonucleotide was removed by twice manipulation of S-400 spin column (Amersham Pharmacia Biotech). After the whole reaction mixture was treated with heat at 25°C for 10 minutes, half of that was electrophoresed with 1% agarose gel. After eletrophoresis, the gel was stained with ethidium bromide, and the photograph of DNA was recorded. Gel was set on filter paper and was dried up in gel dryer. Autoradiogram of the gel was obtained, and signal from labeled oligonucleotide was recorded on X ray film. The result is shown in lane 1 of Figure 6(B).

Please replace the paragraph beginning on Page 33, line 27, with the following:

Figure 6C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide. Intensity of the signals from labeled oligonucleotide from lane 1 to lane 23 were measured with BAS2000 Image analyzer and the result is shown in Figure 7.

Please replace the paragraph beginning on Page 34, line 2, with the following:

We examine the effect of the type of a nucleotide mutation in the oligonucleotide on the formation of triple strand DNA. The same reaction was conducted as lane 1 of Figure 6B in Example 5 except using oligonucleotide 3 and PCR Product (a) as a target DNA. The result is shown in lane 1 of Figure 9B. PCR reaction to prepare PCR Product (a) was conducted using 35-mer primer 1 that has the sequence same as the terminal sequence produced by cutting pBR322 DNA with reaction enzyme Sca I and 35-mer primer 2 that has the sequence of another terminal of the DNA as primers and 1ng pBR322 DNA as template with 27 cycles of 98°C for 20 seconds and 68°C for 5 minutes following the general method. The part of the PCR product was electrophoresed with 1% agarose gel. The PCR Product (a) was extracted using QIAGEN Gel Extraction Kit and purified following the general method.

Please replace the paragraph beginning on Page 34, line 16, with the following:

Figure 9C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide. The nucleotide sequence of the oligonucleotides used were as follows:

Please replace the paragraph beginning on Page 35, line 10, with the following:

The effect of the position of a nucleotide mutation in oligonucleotide to the target DNA on the sensitivity of detection of SNP was examined. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as a target DNA and oligonucleotide that has terminal sequence of the target DNA and has a substituted nucleotide were prepared. Then, the effect of the position of a nucleotide mutation in oligonucleotide on the sensitivity of detection was examined. The result is shown in Figures 10A-10C.

Please replace the paragraph beginning on Page 36, line 31, with the following:

The result is shown in Figures 12A-12C. It is revealed that the detection of SNP is possible regardless of the types and the position of the mutation.

Please replace the paragraph beginning on Page 37, line 1, with the following:

M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as a target DNA and 60 mer oligonucleotide 1 that has terminal sequence of the target DNA were prepared. 5'-terminal of the oligonucleotide was labeled with ^{32}P . The reaction mixture

contains 1 pmol labeled oligonucleotide 1,200 ng target DNA, 3.0 μ g RecA protein, 4.8 mM ATP- γ S, 30 mM Tris acetate (pH 7.2), and 20 mM magnesium acetate. After the reaction mixture was incubated at 37°C for 30 minutes, 0.5 % (W/Vol) SDS and 0.7 mg/ml proteinase K was added to the mixture. Then, the mixture was incubated at 37°C for 30 minutes. After phenol-chloroform extraction was performed once, unused oligonucleotide was removed by twice manipulation of S-400 spin column (Amersham Pharmacia Biotech). After the whole reaction mixture was treated with heat at 25°C for 10 minutes, half of that was electrophoresed with 1% agarose gel. After electrophoresis, the gel was stained with ethidium bromide, and the photograph of DNA was recorded. Gel was set on filter paper and was dried up in gel dryer. Autoradiogram of the gel was obtained to detect signal which was recorded on X ray film. The result is shown in lane 1 of Figure 13B.